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GCMS analysis & assessment of antimicrobial potential of rhizospheric Actinomycetes of AIA3 isolate

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Plants have been used for medicine to support human health in many regions in the world by researchers since ancient times. Plants and soil organisms have been found to have very high therapeutic potential as they produce many natural products. Evolving drug resistance towards nearly all anti-infection drugs, lead to the fast development of new drugs. Many natural products or secondary metabolites have been used for animal and human health. Recently, many new secondary metabolites from actinomycetes have been isolated and reported as important compounds with different activities like anti-microbial, anti-oxidant, anti-inflammatory, anti-androgenic and anticancer agents, etc. In this study isolation of actinomycetes was carried out on actinomycetes isolation agar media (AIA). Characterization and biochemical tests were performed and followed by fermentation and solvent extraction by four solvents for example- Benzene, pet ether, ethyl acetate, chloroform. GCMS was performed for identification of compounds present in culture broth. Major compounds present were Octanal,Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl), Dibutyl phthalate, N-hexadecanoic acid, 1-nonadecene, Heptadecane, Octadecanoic acid, 3,7,11,15-tetramethyl-2-hexadecene, Dihydroergotamine, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecanoic acid, 2,3-dihydroxypropyl ester, 13-docosenamide, and 4-tert-butylcalix[4]arene. Crude obtained was checked for their antimicrobial activity and inhibition zones (IZ) were noted on Mullar Hinton agar (MHA) media against indicator organisms like *Staphylococcus aureus* (MTCC-3160) (IZ=Ben-18 mm, E.A-25 mm), *Pseudomonas aeruginosa* (MTCC 1688) (IZ=Ben-11 mm, Chl-14 mm, E.A-24 mm), *Klebsiella pneumonia* (MTCC-432) (IZ=Ben-19 mm, Chl-20 mm, E.A-34 mm), *Proteus vulgaris* (MTCC-7306) (IZ=Benzene-10 mm, E.A-30 mm), *Bacillus subtilis* (MTCC-441). Identification of compounds was carried out by NIST 14 library.

Keywords: Actinomycetes, Antibacterial, Bioactive compounds, Inhibition zones, Solvents

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In nature, various ecological interactions occur; they can be either negative or positive means for the living beings involved[1]. The rhizosphere is one of the most complex and dynamic microbial living habitat on the earth. In the rhizospheric region, plants and microbes work together and make a special biological community; this incorporates carbon and water cycling, supplement and mineral trapping etc. Considering the plant-organism collaborations, their co-metabolism creates a wide scope of metabolites, which are of incredibly important and are generally known to encourage different purposes including energy sources and signaling components[2]. Secondary metabolites depict a diverse, low molecular weight and complex structural compounds. Microorganisms specifically are productive anti-toxin factories, and have been considered as great potent source of bioactive metabolites[3]. Microbial bioactive components are worldwide known to have good biological activity for human and animal wellbeing. Secondary metabolites are useful as anti-toxins, different medicinal toxins, pesticides, and animal and plant development factors[4]. An ecological niche is a composition of microhabitat that has microscopic structural variety which incorporates microorganisms, protozoa, parasites, nematodes, and a macroscopic variety that incorporates plants and insects. In nature, all organisms need to compete so as to get by in their habitat. This natural biological work can be accomplished by the advancement of competitive mechanisms, for example, the production of toxins, enzymes and antimicrobial components like

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antibiotics. Anti-infection agents or antibiotics are natural or chemically modified substances that can demonstration by repressing growth or development or by killing the cell. These compounds act in distinctive ways. Generally they interfere with the biological activity of organisms, for example, replication, transcription and cell wall synthesis[1]. The microorganisms present in rhizospheric region help plants in development, promotion and yield. Actinomycetes are one of the significant components of rhizospheric microbial populations and are helpful in soil nutrient cycling, plant growth promotion (PGP) and clinical useful antibiotic production. Actinomycetes are a group of aerobic, branched, unicellular gram positive microorganisms and can be found freely or saprophytically in various natural surroundings, for example, soil, warm water, and marine residue with high level of GC (70%) in their hereditary material[5,6]. Actinomycetes can also produce, lytic proteins, PGP substances and important antibiotics and many other secondary metabolites. Most research on secondary metabolites has concentrated on microscopic organisms, essentially soil actinomycetes with most of components have been extracted from the Streptomyces[4]. The actinomycetes, primarily those belonging with Streptomyces sp., make up a large significant group of soil microorganisms and excellent in the degradation of complex molecules to basic or simple substances for plant development and promotion and control plant pathogens[7]. Among all known microbial antibiotics and bioactive metabolites (together with 22,500), at least 45% are only from actinomycetes, 38% are from fungus group and 17% are from unicellular bacteria. Among this abundance of compounds, just about a hundred are practically used for human treatment, with the majority being isolated from actinomycetes[4]. Natural sources have given new synthetic compounds and continued to be a great source for dynamic active substance. There are as yet unexplored or under investigated regions, which need to be investigated for novel bioactive compounds[8]. Because of emergence and re-emergence of drug resistant pathogenic microbes, there is necessity to extend extra potent antimicrobial metabolites with wide spectrum antimicrobial activity. Hence, the actinomycetes were cultured for the production of antimicrobial metabolites using actinomycetes isolation agar (AIA) media.

Materials and methods

Rhizospheric Sample collection

The rhizospheric soil samples were collected from four different locations (Kota, Jaipur, Alwar, and Udaipur) of Rajasthan, India. The debris present in soil samples were removed before collection. Samples were collected in sterile covers from above mentioned four locations. The site was digged into 12–15 cm and approximately 15-20 g of the rhizospheric soil was collected in a sterile cover and transported to Research laboratory of Jaipur Engineering College and Research Centre (JECRC) University, Jaipur, Rajasthan. Rhizospheric soil was oven dried for 24-48 h due to presence of moisture. Samples were processed and then stored at 4^{o}C.

Isolation and maintenance of soil bacteria

The actinomycetes strain AIA3 was isolated from rhizospheric soil. 1 g of each dry soil sample was suspended in 9 mL of sterile distilled water and shaken well. Diluted aliquots (0.2 mL) of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were spread on the plates. The autoclaved actinomycetes isolation agar (AIA) medium containing petri-plates were prepared aseptically so that it minimizes the fungal and bacterial contaminations. Dilutions were spread with a 'L' shaped glass spreader containing actinomycetes isolation agar (AIA)[9] and Plates were incubated inverted at 37^{o}C for 5-7 days. Immediate after the incubation period, morphologically dissimilar colonies were picked up from the petri-plates and re-streaked every colony in suitable media and pure cultures were obtained and stored for further testing[10,11].

Morphological characterization of isolated bacteria

Morphological characterizations of the isolated bacteria were carried out using gram's staining method. Twenty four hours old Luria broth culture of isolate was used for gram's staining followed by biochemical characterization[8]. (Table 1)

Primary screening

Indicator strains

Primary screening of the isolated strain was done *in vitro* against indicator bacterial Cultures. Indicator organisms were *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC-1688), *Klebsiella pneumonia* (MTCC-432), *Proteus vulgaris* (MTCC-7306), *Bacillus subtilis* (MTCC-441), brought from CSIR-IMTECH Chandigarh. These cultures were studied in the Research Laboratory of Jaipur

Table 1 — Characterization of AIA3 isolate

Characteristics	Result
Gram staining	+ve
Aerobic growth	+ve
Anaerobic growth	-ve
Sugar fermentation test	
Starch hydrolysis	+ve
Triple sugar iron (TSI) agar	+ve
Casein hydrolysis	+ve
Citrate Utilization	-ve
Methyl red	-ve
Voges-Proskauer	-ve
Nitrate reduction	-ve
Indole production	-ve
Catalase test	+ve
Other tests	
Urease production	+ve
Hydrogen sulphide production (H_2S)	-ve
Motility test	-ve
Carbon utilization	
Mannitol	-ve
Lactose	-ve
Dextrose	-ve
Sucrose	+ve

Engineering College and Research Centre (JECRC) University Jaipur, Rajasthan. The screening was done by both disc diffusion and agar well method[12,13]. (Fig. 1). These pathogenic strains were maintained in the Nutrient agar media slants for routine laboratory use and stored for the long term usages[14].

Fermentation of actinomycetes and extraction of antimicrobial metabolites

Luria broth was used as the base for the production of the antimicrobial metabolites. Around, 500 mL of the broth was prepared and distributed 500 mL into two Erlenmeyer flasks, 250 mL in each flask and sterilized both flasks. After sterilization, flasks were inoculated with culture and kept in the shaker incubator at 150 rpm at 30°C for two weeks. Immediate after incubation period is over, the cell suspension was centrifuged at 5,000 rpm for 20 min to separate the supernatant and the biomass. Four solvents were used as Pet ether, Benzene, Ethyl acetate and Chloroform for compounds extraction from AIA3 culture broth. Solvent and broth containing isolate was mixed to 1:1 (v/v) quantity and shaken well. Further they kept without interruption for 30 min till two isolate layers got completely separated from each other[13,15]. Solvent beakers (containing bioactive metabolites) were kept at 60°C on water bath for complete evaporation so that we can have only metabolites remain in the container[15]. Crude antimicrobial metabolites were extracted using four solvents with the help of separating funnel. Mixture of bioactive metabolites was transferred to small eppendorf tube for further Gas Chromatography Mass Spectroscopy (GCMS) study[13,16].

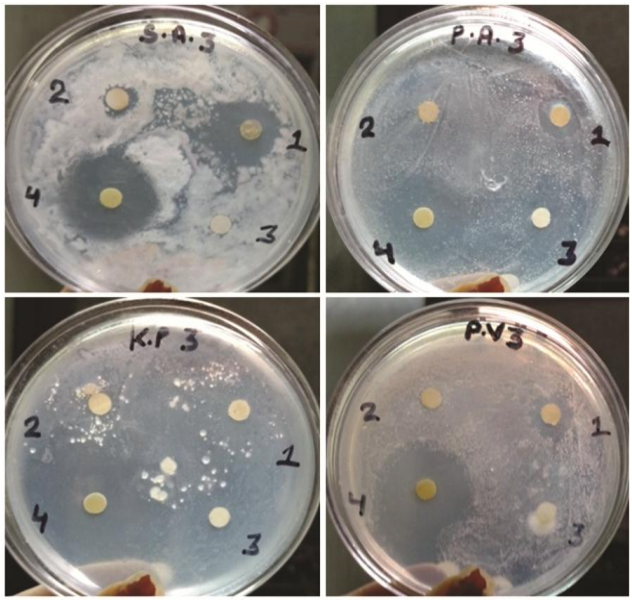

Fig. 1 — Inhibition zones (IZ) of Antibacterial susceptibility test of AIA3 isolate against test pathogens.

Inhibition zones (IZ) of antibacterial activity against indicator pathogens

Inhibition zones (IZ) of activity of the crude extracts of benzene, pet ether, ethyl acetate, chloroform was performed against *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC-1688), *Klebsiella pneumonia* (MTCC-432), *Proteus vulgaris* (MTCC-7306), *Bacillus subtilis* (MTCC-441) indicator pathogens. Standard Kirby-Bauer disc diffusion method and agar well diffusion methods were used for antimicrobial activity of crude extracts on petri plates containing 20 mL of Muller Hinton Agar (MHA) media. The indicator strains were swabbed on the solidified MHA media and hold for 10 min for drying of plates. The tests were conducted with each crude extracts. The sterilized discs with crude were placed on the MHA media and kept it for 30 min at normal room temperature. So that complete diffusion of compounds can be done. The plates were incubated over night at 37°C and Inhibition zones (IZ) were measured (mm) against each pathogen[17,18]. (Fig. 1, Table 2, Fig. 1A)

Identification of main bioactive components present in Gas Chromatography-Mass Spectrometry (GC–MS) analysis.

The composition of the active extracts of AIA3 isolate was determined by GCMS analysis using

Table 2 — Inhibitions zones (IZ) of antibacterial activity of AIA3 Isolate against different Test pathogens

Test Pathogens Solvents	*Staphylococcus aureus* (MTCC-3160)	*Pseudomonas aeruginosa* (MTCC 1688).	*Bacillus subtilis* (MTCC 441)	*Klebsiella Pneumonia* (MTCC 432)	*Proteus vulgaris* (MTCC 7306)
Benzene	18 mm	11 mm	-	19 mm	10 mm
Pet ether	-	-	-	-	-
Chloroform	-	14 mm	-	20 mm	-
Ethyl acetate	25 mm	24 mm	-	34 mm	30 mm

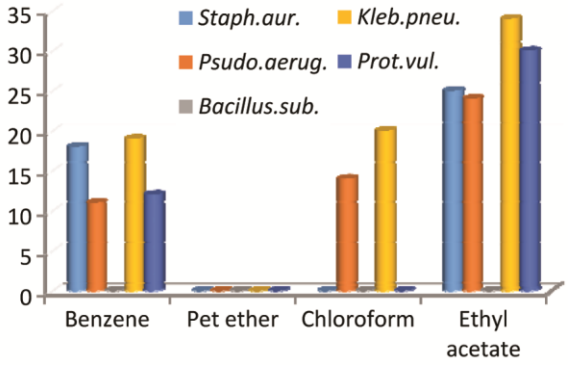

Fig. 1A — Graphical representation of Antibacterial activity of AIA3 isolate against five test pathogens

Shimadzu QP2010 ultra and gas chromatograph interfaced to a mass spectrometer GC–MS. The instrument was built with Elite-1 fused silica capillary. Helium gas (99.9%) was used as the carrier gas and with the flow rate of 1.21 mL/min. Helium gas (99.99%) was the carrier gas with a constant flow rate of 1.21 mL/min and with split ratio: 10. Temperature of Injector was 260°C; Ion-source temperature 200°C. The oven temperature was intended from 60°C (constant for 3 min.) with an increment as of 280°C for 22 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds. The chemical composition of the extract was determined by measuring the peak area and the retention time by comparing the NIST 14 library[19].

Results & discussion

Secondary metabolites have been considered the reliable source of therapeutic drugs since ancient time. On the contrary, because of drug resistance emergence of diverse human diseases with the changing environment, continuous screening along with validation of bioactive secondary metabolites in the form of effective drug needs to be updated. Secondary metabolites are potent bioactive components isolated from plants and soil organisms as well. Isolation of actinomycetes was done on Actinomycetes isolation agar (AIA) media. Biochemical test like Starch hydrolysis, Triple sugar iron (TSI) agar, Casein hydrolysis, Citrate Utilization, Methyl red, Voges-Proskauer, Nitrate reduction, Indole production, Catalase test, Urease production, Hydrogen sulphide production (H_2S), Motility test, Mannitol, Lactose, Dextrose, Sucrose were carried out with actinomycetes AIA3 isolate. Isolate was positive in Gram staining, aerobic growth, Starch hydrolysis, Triple sugar iron (TSI) agar, Casein hydrolysis, Catalase test, Urease production and Sucrose test. Characterization of pure colony, broth fermentation and extraction of bioactive components from four solvents like pet ether, benzene, ethyl acetate and chloroform from the soil actinomycetes was performed. For the presence of compounds in isolate were characterized by means of GCMS. Isolate was checked for antimicrobial activity against selected indicator pathogens which was brought from IMTECH Chandigarh. Extraction of components was processed by layer separation technique and sterlized discs with components were applied on prepared plates of Muller Hinton Agar (MHA) media already occupied with pathogenic growth. Antimicrobial susceptibility test was performed against indicator strain *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumonia* (MTCC-432), *Proteus vulgaris* (MTCC-7306), *Bacillus subtilis* (MTCC-441) brought from IMTECH Chandigarh. Major compounds obtained in Ethyl acetate extract of AIA3 isolate were, Octanal with RT-14.353, Pyrrolo[1,2-a]pyrazine-1,4-dione with RT-20.360, hexahydro-3-(2-methylpropyl) with RT- 22.875, Dibutyl phthalate with RT-22.875, N-hexadecanoic acid with RT-22.993, 1-nonadecene with RT-23.342, Heptadecane with RT- 23.418, Octadecanoic acid with RT-25.332, 3,7,11,15-tetramethyl-2-hexadecene with RT-25.737, Dihydroergotamine with RT- 27.314, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester with RT- 29.578, Octadecanoic acid, 2,3-dihydroxypropyl ester with RT- 33.172, 13-docosenamide with RT- 33.893, and 4-tert-butylcalix[4]arene with RT- 54.173. A relative concentration of compounds

was shown by chromatogram of AIA3 isolate. (Fig. 2) Height of every peak is proportional to its present concentration of compounds. Inhibition zones (IZ) of antibacterial activity of crude extracts were recorded against *Staphylococcus aureus* (IZ=Ben-18 mm, E.A-25 mm) *Pseudomonas aeruginosa* (IZ=Ben-11 mm, Chl-14 mm, E.A-24 mm), *Klebsiella pneumonia* (IZ=Ben-19 mm, Chl-20 mm, E.A-34 mm) *Proteus vulgaris* (IZ=Benzene-10 mm, E.A-30 mm) and no activity was recorded against *Bacillus subtilis.* (Table 2, & Fig. 1A) Structure, Molecular weight, Molecular formula, Retention time (RT) and Area percentage of components obtained in GCMS was identified from NIST14 library. Fatty acids (saturated, unsaturated), phenols, terpenes, alkaloids, alcohols, amide compounds etc. were present in good to moderate amount (Table 3).

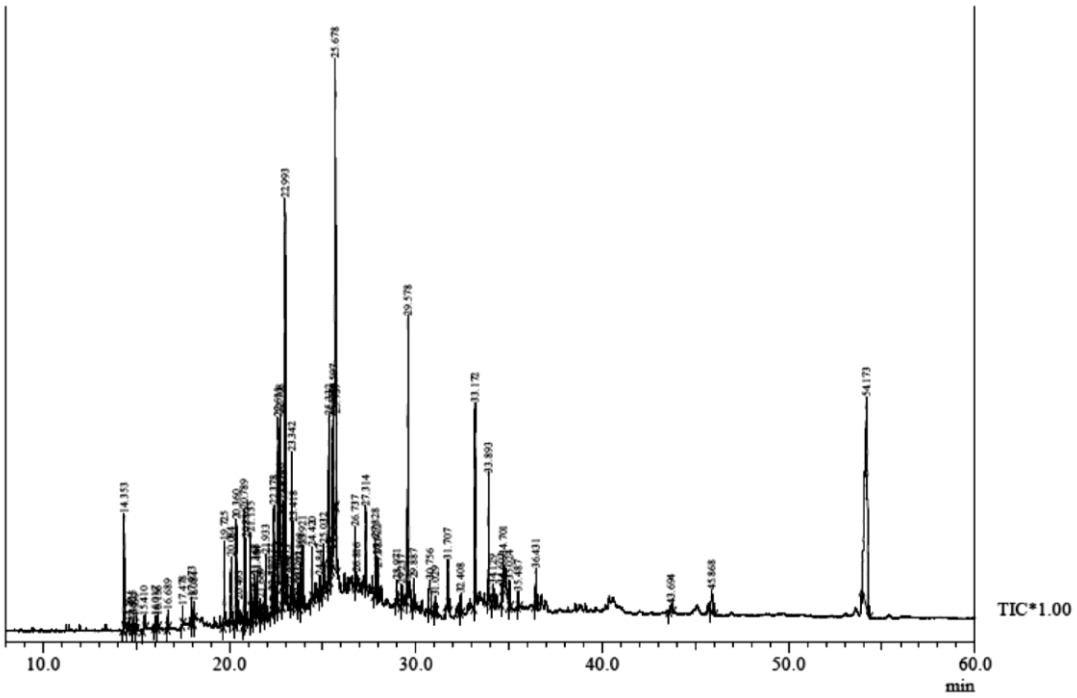

Fig. 2 — Chromatogram of AIA3 isolate showing peaks of compounds in GCMS analysis

Table 3 — Composition of Ethyl acetate extract of rhizospheric AIA3 isolate by GCMS analysis

RT	Compound name	Molecular formula	Molecular weight
14.353	Octanal	$C_8H_{16}O$	128.215
14.567	2,3-dimethylcyclohexanol 1	$C_8H_{16}O$	128.215
14.825	1-tridecene	$C_{13}H_{26}$	182.351
14.959	Tridecane	$C_{13}H_{28}$	184.367
15.410	Benzeneethanol, 4-hydroxy-	$C_9H_{12}O$	136.194
16.017	2,6-di-butyl-2,5-cyclohexadiene-1,4-dione	$C_{14}H_{20}O_2$	220.312
16.156	Hexadecane, 1-chloro-	$C_{16}H_{33}Cl$	260.890
16.689	Acetamide, n-(2-phenylethyl)-	$C_{10}H_{13}NO$	163.220
17.478	Dodecanoic acid	$C_{12}H_{24}O_2$	200.322
17.973	1-pentadecene	$C_{15}H_{30}$	210.405
18.084	Tetradecane	$C_{14}H_{30}$	198.394
19.725	3-methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, n-acetyl-	$C_{10}H_{14}N_2O_3$	210.229
20.360	Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154.169
20.789	1-heptadecene	$C_{17}H_{34}$	238.459
20.880	Nonadecane	$C_{19}H_{40}$	268.529
21.287	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.403
21.368	Tyrosol, acetate	$C_{10}H_{12}O_3$	180.203
21.484	3-isobutylhexahydropyrrolo [1,2-a] pyrazine-1,4-dione	$C_{11}H_{18}N_2O_2$	210.277

(*Contd*.)

Table 3 — Composition of Ethyl acetate extract of rhizospheric AIA3 isolate by GCMS analysis (*Contd*.)

RT	Compound name	Molecular formula	Molecular weight
21.669	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376.537
21.933	1-hexadecanol	$C_{16}H_{34}O$	242.447
22.277	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.376
22.317	Nonane, 5-methyl-5-propyl-	$C_{13}H_{28}$	184.367
22.480	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.457
22.708	2,5-piperazinedione, 3,6-bis (2-methylpropyl)-	$C_{12}H_{22}N_2O_2$	226.320
22.736	5,10-diethoxy-2,3,7,8-tetrahydro-1h,6h-dipyrrolo[1,2-a:1,2-31	$C_{14}H_{22}N_2O_2$	250
22.875	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.348
22.993	N-hexadecanoic acid	$C_{16}H_{32}O_2$	256.430
23.073	Nonadecane, 3-methyl-	$C_{20}H_{42}$	282.556
23.275	9,10-anthracenedione	$C_{14}H_8O_2$	208.216
23.342	1-nonadecene	$C_{19}H_{38}$	266.513
23.418	Heptadecane	$C_{17}H_{36}$	240.475
23.663	Isopropyl palmitate	$C_{19}H_{38}O_2$	298.511
23.702	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.457
23.808	9-octadecenoic acid (z)-	$C_{18}H_{34}O_2$	282.468
23.921	Palmitic acid, tms derivative	$C_{19}H_{40}O_2Si$	328.612
24.420	Carbonic acid, monoamide, n-decyl-, 2-ethylhexyl ester	$C_{19}H_{39}NO_2$	388
24.847	Octadecane	$C_{18}H_{38}$	254.502
25.332	Octadecanoic acid	$C_{18}H_{36}O_2$	284.484
25.413	Tetracosane	$C_{24}H_{50}$	338.664
25.678	Acetamide, n-[2-(1h-indol-3-yl) ethyl]-	$C_{13}H_{16}N_2O$	216.284
25.737	3,7,11,15-tetramethyl-2-hexadecene	$C_{20}H_{40}$	280.540
26.737	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	312.494
26.816	Tetratetracontane	$C_{44}H_{90}$	619.204
27.314	Dihydroergotamine	$C_{33}H_{37}N_5O_5$	583
27.987	Octacosane	$C_{28}H_{58}$	394.772
28.971	Octadecanoic acid, 3-oxo-, ethyl ester	$C_{20}H_{38}O_3$	326.521
29.237	D-ribose, 2-deoxy-bis(thioheptyl)-dithioacetal	$C_{19}H_{40}O_3S_2$	380.509
29.578	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.509
29.887	1,2-benzenedicarboxylic acid	$C_6H_8O_4$	166.132
30.756	L-prolinamide, 5-oxo-l-prolyl-l-phenylalanyl-4-hydroxy-	$C_{19}H_{24}N_4O_5$	388
31.029	N-tetracosanol-1	$C_{24}H_{50}O$	354.663
32.408	Hexadecanoic acid, 2-(acetyloxy)-1-[(acetyloxy) methyl]ethyl ester	$C_{23}H_{42}O_6$	414.576
33.172	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{45}BO_7$	420.394
33.893	13-docosenamide, (z)-	$C_{22}H_{43}NO$	337.592
34.603	Phosphonic acid, dioctadecyl ester	$C_{36}H_{75}O_4P$	602.966
34.701	9-octadecenoic acid (z)-, 2,3-bis (acetyloxy) propyl ester	$C_{25}H_{44}O_6$	440.621
35.024	Eicosanoic acid, 2,3-bis (acetyloxy) propyl ester	$C_{27}H_{50}O_6$	470.691
35.487	Pentatriacontane	$C_{35}H_{72}$	492.961
36.431	9-octadecenamide	$C_{18}H_{35}NO$	281.484
43.694	beta.-sitosterol	$C_{29}H_{50}O$	414.718
45.868	1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-	$C_{20}H_{34}O$	290.490
54.173	4-tert-butylcalix[4]arene	$C_{44}H_{56}$	584.932

Actinomycetes have capability and produces diverse compounds showing a good range activity against variety of indicator pathogen. Octanal is a major component of essential oil found in *Hydnora Africana* having antioxidan t and antimicrobial activity. In traditional medicine, the root of *Hydnora africana* species have been used to treat a variety of human diseases with inflamed throat, tuber, fruits, leaves and fruit pulp is used for the cure of infectious diseases such as dysentery, diarrhoea, amenorrhoea, bladder and kidney complaints[20]. Octanal is also having cytotoxic properties, reported in *Syzygium polyanthum* plant. *Syzygium polyanthum* plant is being traditionally used for the treatment of diseases that include diarrhea, rheumatism, diabetes mellitus, hypercholesterolemia, hypertension, gastritis and

hyperuricemia[21]. Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) compound have antifungal activity and isolated from Streptomyces species[22]. Dibutyl phthalate reported as effective antibacterial compound from plant *Ipomea carnea*. *Ipomea carnea* leaves used in a skin disease in some rural areas of Chhattisgarh, India. There are some findings on synergistic effect of insecticides with plant extracts against malarial Vector Anopheles stephensi[23]. Presence of Hexadecanoic acid, Octadecanoic acid and 13-Docosenamide have been reported in the plant *Clerodendrum phlomidis*. Hexadecanoic acid and Octadecanoic acid have antioxidant, antimicrobial, hypocholesterolemic, antiarthritic, anti-inflammatory activity and 13-Docosenamide have been reported as antimicrobial activity. The plant *Clerodendrum phlomidis* belongs to the family verbenaceae and it has importance as it is medicinally very useful in the treatment of nervous disorders, inflammation, asthma, rheumatism, digestive disorders, urinary disorders and diabetes etc. In clinicthey have been reported as powerful drug having anti-inflammatory, hypoglycemic, immune modulatory, anti-diarrhoeal and anti-plasmodial properties[24]. 1-Nonadecene compound has antifungal activity reported in *Croton bonplandianum* plant. Plant is useful to anticipation of liver infections, ring worms and skin diseases, body swelling[25]. Heptadecane has antifungal activity in *Lepidagathis cristata Willd*. (Acanthaceae). *Lepidagathis cristata* is a medicinal herb and very useful tonic in fevers and in pneumonia, flu, mouth infections, eczema, psoriasis and other skin problems. The ash of whole herb is applied externally on chronic wounds of pet animals. The roots used in stomachic and dyspepsia, leaves are important for fevers[26]. 3,7,11, 15-tetramethyl-2-hexadecene has been known for its antimicrobial, anti-inflammatory activity in *Fluggea leucopyrus plant. Fluggea leucopyrus* Wild. Plant is sweet, cooling, diuretic, aphrodisiac, tonic useful in vitiated conditions of pitta, burning sensation, strangury, seminal weakness and general debility. Its leaves act as a anti-infection and its paste is useful to extract any extraneous substance from tissues without surgery. Paste of its leaves if mixed with tobacco is used to destroy worms in sores[27]. Dihydroergotamine works as anti-migraine therapy. It is useful in acute Migraine problem and used as vasopressor[29,30]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester *have* Hemolytic, pesticide, flavour, antioxidant activity isolated from *Pistia stratiotes* L. Leaves of *Pistia stratiotes* are traditionally used against ringworm infection of scalp, boils and syphilitic eruptions. Traditionally, its oil extracts is useful for the treatment of tuberculosis, asthma and dysentery. *Eichhornia crassipes* plant is also useful for medical purpose[31]. Octadecanoic acid, 2,3-dihydroxypropyl ester have strong anti-microbial and anti-cancerous compound isolated from *Cenchrus biflorus* plant[32]. 4-tert-butylcalix [4] arene compound have been reported as allelopathic suppression in *Cassia tora L*. and mitotic changes on *Allium cepa L*[33] (Table 4) .

Table 4 — Major Components present in Ethyl acetate extract of isolate and their biological activity

RT	Name of Compound	Nature of compound	Other Sources of compounds	Parts used	Activity
14.353	Octanal	Saturated fatty aldehyde	*Hydnora .africana* *Sysygium polyanthum*	Roots, Tuber, fruits, Leaves & Fruit pulp Leaves	Treatment of infectious Diseases such as dysentery, Diarrhea, amenorrhoea, bladder & kidney[20] Cytotoxicity[21]
20.360	Pyrrolo[1,2-a]pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl)	Pyrrolizidine	*Streptomyces*	Ethyl acetate extract	Antifungal & Antibacterial[22]
22.875	Dibutyl phthalate	Colorless oily liquid	*Ipomoea carnea*	Stem	Antibacterial[23]
22.993	N-hexadecanoic acid	Saturated long chain fatty acid	*Clerodendrum phlomidis L.* (*Verbenaceae*)	Leaves	Anti-oxidant Nematicide[24]
23.342	1-nonadecene	Unbranched alkene	*Croton bonplandianum* (Euphorbiaceae family	Leaves	Antifungal[25]
23.418	Heptadecane	Straight chain alkane	*Lepidagathis cristata* Willd. (Acanthaceae)	Leaves	Antifungal[26]

(*Contd*.)

Table 4 — Major Components present in Ethyl acetate extract of isolate and their biological activity (*Contd*.)

RT	Name of Compound	Nature of compound	Other Sources of compounds	Parts used	Activity
25.332	Octadecanoic acid	Straight chain saturated fatty acid	*Pterocarpus angolensis*	Stem bark	Antimicrobial Activity[24]
25.737	3,7,11,15-tetramethyl-2-hexadecene	Terpene alcohol	*Fluggea leucopyrusWilld. (Euphorbiaceae)*	Aerial parts	Antimicrobial, Anti-inflammatory[27]
27.314	Dihydroergotamine	Alkaloid	*Pseudomonas aeruginosa*	Methanolic-exract	Unkonwn activity[28] Anti Migraine therapy[29] Vasopressor[30]
29.578	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Amino compound	*Pistia stratiotes* L. & *Eichhornia crassipes*	Leaves	Hemolytic, pesticide, flavour, antioxidant[31]
33.172	Octadecanoic acid, 2,3-dihydroxypropyl ester	Fatty acid	*Cenchrus biflorus Roxb*	Leaves	Anticancer, antimicrobial[32]
33.893	13-docosenamide, (z)-	Amino compound	*Clerodendrum phlomidis L. (Verbenaceae)*	Leaves	Anti-microbial[24]
54.173	4-tert-butylcalix[4]arene	Peptides	*Nicotiana plumbaginifolia*	Stem	Allelopathic suppression in *Cassia toraL*. and mitotic changes on *Allium cepa L*[33].

Conclusion

The present study concluded that rhizospheric soil actinomycetes possess strong antimicrobial activity against the indicator pathogens. The extracellular components secreted by the actinomycetes isolate were extracted using organic solvents which were present and displayed antimicrobial activity. GCMS studies have demonstrated the presence of variety of group of bioactive compounds at different concentration level. Inhibition zones (IZ) of antimicrobial activity of the extracts of actinomycetes isolate against the drug resistant clinical pathogens were comparable with the other researcher's studies. On the whole, the microorganism recovered from unexplored rhizospheric regions of Rajasthan was the promising source for the revival of secondary metabolites with broad level of activities which guided for the development of new antibiotics.

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